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ORIGINAL RESEARCH

A Potential Role for the STXBP5-AS1 Gene in Adult ADHD Symptoms

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Abstract

We aimed to detect Attention-deficit/hyperactivity (ADHD) risk-conferring genes in adults. In children, ADHD is characterized by age-inappropriate levels of inattention and/or hyperactivity-impulsivity and may persists into adulthood. Childhood and adulthood ADHD are heritable, and are thought to represent the clinical extreme of a continuous distribution of ADHD symptoms in the general population. We aimed to leverage the power of studies of quantitative ADHD symptoms in adults who were genotyped. Within the SAGA (Study of ADHD trait genetics in adults) consortium, we estimated the single nucleotide polymorphism (SNP)-based heritability of quantitative self-reported ADHD symptoms and carried out a genome-wide association meta-analysis in nine adult population-based and case-only cohorts of adults. A total of n = 14,689 individuals were included. In two of the SAGA cohorts we found a significant SNP-based heritability for self-rated ADHD symptom scores of respectively 15% (n = 3656) and 30% (n = 1841). The top hit of the genome-wide meta-analysis (SNP rs12661753; p-value = 3.02×10^{-7}) was present in the long non-coding RNA gene *STXBP5-AS1*. This association was also observed in a meta-analysis of childhood ADHD symptom scores in eight population-based pediatric cohorts from the Early Genetics and Lifecourse Epidemiology (EAGLE) ADHD consortium (n = 14,776). Genome-wide meta-analysis of the SAGA and EAGLE data (n = 29,465) increased the strength of the association with the SNP rs12661753. In human HEK293 cells, expression of *STXBP5-AS1* enhanced the expression of a reporter construct of *STXBP5*, a gene known to be involved in "SNAP" (Soluble NSF attachment protein) Receptor" (SNARE) complex formation. In mouse strains featuring different levels of impulsivity, transcript levels in the prefrontal cortex of the mouse ortholog *Gm28905* strongly correlated negatively with motor impulsivity as measured in the five choice serial reaction time task ($r^2 = -0.61$; $p = 0.004$). Our results are consistent with an effect of the *STXBP5-AS1* gene on ADHD symptom scores distribution and point to a possible biological mechanism, other than antisense RNA inhibition, involved in ADHD-related impulsivity levels.

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A. B. Smit, B. Franke and D. I. Boomsma shared final responsibility.

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Extended author information available on the last page of the article

Keywords ADHD symptoms · GWAS · Adults · *STXBP5-AS1* gene

Introduction

Attention-deficit/hyperactivity disorder (ADHD) is a common neurodevelopmental disorder affecting 2–5% of children (Polanczyk and Rohde 2007) and 2.5–4.9% of adults (Franke et al. 2018). In children, ADHD is characterized by age-inappropriate, sustained symptoms of inattention and/or hyperactivity-impulsivity. In children and adults (Faraone et al. 2005) ADHD shows substantial heritability. Heritability (h^2) estimates are largely independent of the phenotypic measurement scale (i.e., categorical or continuous) and estimates are lower when using self-report rating scales (Franke et al. 2012). For clinically diagnosed ADHD, the genetic contribution to variation in ADHD was estimated at 72% in adult twins from the Swedish Twin Registry (n = 37,714) (Larsson et al. 2014), whereas the estimate was around 30% for the self-assessed ADHD index scored from the Conners' Adult ADHD Rating Scales in a large (n = 12,594 subjects) Dutch sample of twins and their relatives (Boomsma et al. 2008).

For Attention Problems assessed by ASEBA (Achenbach System of Empirically Based Assessment; Achenbach et al. 2003) in 44,607 young and adult Dutch twins (57% with longitudinal data), there was a downward trend with age. Age to age correlations were age dependent and ranged from 0.33 (age 50–60+) to 0.73 (age 10–12). The stability across ages was explained by both genetic and environmental factors. Heritability at age three was 70% and remained high before age 12 (72–74%). After age 12, h^2 became 50–56% in adolescents, and this declined further to 40–54% in adults (Kan et al. 2013). However, part of the decrease in h^2 may not be due to age but to differences in assessment (Kan et al. 2013, 2014).

A large Swedish study addressed the etiology of the association of the major dimensions of ADHD in adults and estimated that 52% of the correlation between inattentive and hyperactive-impulsive symptoms was accounted for by genetic influences, and 48% by non-shared environmental influences (n = 15,198 adult twin pairs) (Larsson et al. 2013). Shared genetic factors also underlie the increased risks for other psychiatric disorders adults with ADHD and their relatives, for example borderline personality disorder (Distel et al. 2011; Kuja-Halkola et al. 2018) autism spectrum disorder (Ghirardi et al. 2018) and problem drinking (Derks et al. 2014). However, not all comorbidities are due to genetic risk; for the liability of attention-deficit/hyperactivity and tics and obsessive–compulsive symptoms, the majority of genetic variance was specific rather than shared (Pinto et al. 2016).

The genetic contributions to ADHD in children and adults are complex, with multiple different genetic variants contributing to the disorder (Faraone et al. 2015), both common and rare (Franke et al. 2012). Recently, 16 genome-wide associations have been established in an ADHD Genome-Wide Association Studies meta-analysis (GWASMA) of childhood case-control studies from the psychiatric genomics consortium (PGC) and The Lundbeck foundation initiative for integrative psychiatric research (iPSYCH) (Demontis et al. 2018) and population-based samples from the early genetics and lifecourse epidemiology (EAGLE) consortium (Middeldorp et al. 2016). These studies estimated that the single nucleotide polymorphism (SNP)-based h^2 of ADHD symptom scores in children ranged from 5 to 34% in population-based samples (EAGLE consortium) and was 21.6% in the PGC + iPSYCH case-control samples.

Inattention and hyperactivity–impulsivity symptoms can be reliably assessed in population-based cohorts based on rating scales. Phenotypic and genetic correlations between symptom scores assessed with the different instruments are substantial: in a clinical sample of 120 adults with ADHD the phenotypic correlation between the CAARS ADHD-index (Conners 1999) and the ADHD-RS, which obtains ADHD DSM-IV symptoms self-report was 0.73 ($p < 0.01$) (Kooij et al. 2005). In 380 parents of children with ADHD, this correlation was of similar magnitude (r = 0.69; $p < 0.001$) (Thissen et al. 2012).As such, the disorder may be viewed being at the extreme end of normally distributed behavioral traits in the population (Larsson et al. 2013). This creates the possibility to collect large samples for gene-finding studies.

Here, we aimed to identify genes associated with ADHD symptoms in adults from population-based and case-control cohorts in order to detect disease-relevant genes. Within the study of ADHD trait genetics in adults (SAGA) consortium, we estimated the SNP-based h^2 of self-reported adult ADHD symptoms and subsequently carried out a GWASMA in nine cohorts of European-Caucasian origin (n = 14,689 individuals, age 18 years or older). These samples included six population-based cohorts, two clinical ADHD samples and one clinical cohort ascertained for depressive and anxiety disorders. We followed up the locus with the strongest statistical association in a replication analysis of quantitative childhood ADHD symptom scores (n = 14,776) from the EAGLE consortium (Middeldorp et al. 2016). Genetic correlations were estimated between the SAGA sample and the PGC + iPSYCH sample of children (Demontis et al. 2018) and between SAGA and the ADHD GWASMA results of the EAGLE consortium (Middeldorp et al. 2016). Finally, we conducted gene-based tests for genes with SNPs showing

a p-value $< 1 \times 10^{-6}$ in the meta-analysis, making use of the common SNPs from SAGA and rare variant data from the Erasmus Rucphen Family (ERF) study (see Table 1), included in SAGA.

Functional follow-up studies downstream of gene finding in ADHD, e.g. in model systems, to determine the biological relevance of a genetic finding are scarce (Klein et al. 2017). Core features of ADHD, inattention, hyperactivity, and impulsivity are well defined in mouse models (Loos et al. 2014). Here we carried out functional follow-up studies for the gene with the top association result from the GWASMA in three mouse inbred strains with large differences in motor impulsivity derived from reaction time tasks, and in a human cell assay.

Methods

Study populations in the SAGA consortium

ADHD symptom scores in adults were available in nine cohorts of European descent (please see references in Table 1; Supplementary Table 1; Supplementary Fig. 4).

(1) The Netherlands Twin Registry (NTR) is a population-based longitudinal cohort of twins and their family members, e.g. parents, siblings, spouses, which recruited (young) adult twins in the early 1990s through city council registrations. Continued recruitment of adult twins took place continuously via the NTR website and e.g. via media campaigns. Twin families are followed longitudinally through survey studies and biobank projects.
(2) The Netherlands Study of Depression and Anxiety (NESDA) is an ongoing longitudinal naturalistic cohort

Table 1 Information on cohorts and phenotype assessment in the SAGA consortium included in the GWASMA

Cohort name	N (% F)	Age (SD)	Symptom list (N items)	Score range[a]	Mean score (SD)[a]	Sample type	References
Netherlands twin registry (NTR)	5935 (63%)	43.7 (15.2)	CAARS ADHD-index (12)	0–30	7.9 (3.7)	Population-based	Willemsen et al. (2010)
Netherlands study of depression and anxiety (NESDA)	1977 (66%)	46.5 (13.0)	CAARS ADHD-index (12)	0–32	8.7 (5.4)	Clinical population of major depression disorder	Boomsma et al. (2008)
Erasmus rucphen family study (ERF)	1043 (53%)	45.6 (13.3)	CAARS ADHD-index (12)	0–25	7.8 (4.4)	Population-based	Aulchenko et al. (2004)
NeuroIMAGE	470 (51%)	42.3 (5.3)	ADHD-RS (23)	0–43	14.1 (8.9)	Parents of children of ADHD	von Rhein et al. (2014)
Brain imaging genetics (BIG)	448 (63%)	22.3 (3.2)	ADHD-RS (23)	0–40	14.0 (6.4)	Population-based	Hoogman et al. (2012)
Nijmegen biomedical study (NBS)	2925 (53%)	57.4 (16.3)	ADHD-RS (23)	0–15	1.4 (2.2)	Population-based	Galesloot et al. (2017)
(International multicentre persistent ADHD collaboration IMpACT)[b]	113 (63%)	37.7 (11.5)	ADHD-RS (23)	1–18	12.04 (3.3)	Clinical population of adults with ADHD	Mostert et al. (2015)
Vall d'Hebron Institute de recerca (VHIR)[b]	559 (32%)	33.3 (10.6)	ADHD-RS (18)	4–54	31.0 (9.7)	Clinical population of adult ADHD	Richarte et al. (2017)
TRacking adolescents' individual lives survey (TRAILS)	1215 (48%)	19.0 (0.6)	ASR ADHD (13)	0–26	5.9 (4.4)	Population-based	Oldehinkel et al. (2015)

F female, *CAARS ADHD-index* Conners' Adult ADHD Rating Scale, *ADHD-RS* DSM-IV ADHD Rating Scale, *ASR ADHD* Attentional Deficit/Hyperactivity Problems subscale from the Adult Self Rating

[a]Untransformed values observed per cohort

[b]Only affected individuals included

study of 2981 people, aged 18–65 years at baseline, with lifetime and/or current depressive and/or anxiety disorders (n = 2329, 78%) and healthy controls (n = 652, 22%). Participants were recruited from the community (n = 564, 19%), primary care (n = 1610, 54%) and specialized mental healthcare (n = 807, 27%) from September 2004 to February 2007 at three study sites (Amsterdam, Groningen, Leiden). Exclusion criteria were: (a) having a primary clinical diagnosis of psychotic disorder, obsessive–compulsive disorder, bipolar disorder or severe addiction disorder, and (b) not being fluent in Dutch.

(3) The TRacking Adolescents' Individual Lives Survey (TRAILS) is an ongoing, multidisciplinary research project on the psychological, social and physical development of adolescents and young adults. More than 2500 young people participate, since their tenth or eleventh year of age. These participants have been examined every 2–3 years for the past 15 years, through questionnaires, interviews, tests and/or physical measurements. Information is provided by youngsters, family members, teachers and partners.

(4) Erasmus Rucphen Family (ERF) is a family-based cohort originating from 22 couples and spread over 23 generations. All descendants of those couples were invited to visit the clinical research center in the region, where they were examined in person and interviewed on a broad range of topics, including medication use and medical history.

(5) International Multicenter ADHD Genetics (NeuroIMAGE) collected DNA and information on ADHD and relevant comorbidities from families with (at least) one child diagnosed with ADHD between 2003 and 2007. The Dutch site of the IMAGE project also collected other (cognitive and behavioral) measures on (unaffected) parents. The behavioral information on the parents was used in this study.

(6) The Brain Imaging Genetics (BIG) study is a population-based study of healthy individuals (age range 18–45 years) who participated in imaging studies carried out in the Donders Institute, in Nijmegen, the Netherlands. The assessment of ADHD symptoms was performed through internet-based testing, as part of an electronic questionnaire and test battery.

(7) The Nijmegen Biomedical Study (NBS) is a population-based study that was initiated in 2000 in Nijmegen in the eastern part of The Netherlands. Eligibility criteria were age 18 years or older, not living in an institution or rest home and the ability to fill out a questionnaire in Dutch. Participants were invited to fill out a postal questionnaire (NBS-1 QN) and to donate a blood sample. Psychological problems and symptoms for ADHD were collected in the second wave of the study (NBS-2), that was initiated in 2005.

(8) The International Multicenter persistent ADHD CollaboraTion (IMpACT) included patients and healthy controls recruited at the department of psychiatry, Radboud University Medical Center, Nijmegen and through advertisements. Patients were included if they had previously been diagnosed with persistent ADHD, i.e. present since childhood, by a psychiatrist according to the DSM-IV-TR. Exclusion criteria were psychosis, addictions in the last 6 months, current Major Depression Disorder, full-scale IQ estimate < 70, neurological disorders, sensorimotor disabilities, medication use other than psychostimulants, atomoxetine or bupropion and failure to withhold stimulant medication 24 h prior to testing. These same criteria were applied to the NeuroIMAGE sample.

(9) The Vall d'Hebron institute de recerca (VHIR) sample consisted of ADHD cases only. Participants were referred to the program from primary care centres, children's neuropsychiatric surgeries or mental health hospitals in Barcelona, Spain because of a diagnostic suspicion of ADHD. The diagnosis of ADHD was evaluated with the structured clinical interview for DSM-IV (SCID-I) and the conners adult ADHD diagnostic Interview for DSM-IV (CAADID-II).

ADHD symptom scores in the SAGA consortium

ADHD symptom scores were assessed by three instruments (see Table 1). First, the ADHD-index of the Conners Adult ADHD Rating Scale (CAARS ADHD-index) (Conners 1999) which consists of 12 items, scored on a 4-point scale (0 = never, 3 = very often; see Supplementary Table 8 for details). The CAARS ADHD-index (used in NESDA, NTR, ERF) is an extensively tested psychometric instrument with high internal consistency and reliability.

Second, the total scores of the DSM-IV ADHD Rating Scale (ADHD-RS) (Kooij et al. 2005). Five cohorts (NeuroIMAGE, BIG, IMpACT, VHIR, NBS) collected information using the ADHD-RS (Kooij et al. 2005), which has high validity in population-based and case samples. Subjects were asked to complete the ADHDRS for current symptoms in adults. Symptoms were reported over the last 6 months. Participants had to answer 23 questions on a four-point scale (never, sometimes, often, very often). The 23 current item scores were recalculated to the original 18 DSM-IV-TR ADHD criteria.

Third, the Attentional Deficit/Hyperactivity Problems subscale from the adult self report (ASR ADHD; 13 items) (Achenbach et al. 2003) was used by one cohort, TRAILS. The ASR ADHD scale has 13 items; response format is 0, 1, and 2 per item. Possible range in scores 0–26. In TRAILS,

GWAS analyses were done with the phenotype measured at the level of the item average rather than the item sum.

Genetic variant calling and quality control in the SAGA consortium

An overview of genome-wide SNP genotyping, quality control, and imputation is given in Supplementary Table 1. Exomes of 1336 individuals from the ERF population, which is a genetically isolated population in the Netherlands (Aulchenko et al. 2004), were sequenced (see Supplementary Methods), and ADHD index data were available for 587 of these individuals. Detection of rare variants in the ERF study was done for genes harboring SNPs with association $p < 1 \times 10^{-5}$ in the GWASMA, and variants identified in these exomes were used to estimate the contribution of rare variants in the genes of interest to ADHD behavior (see Supplementary Methods).

GCTA

Genome-wide Complex Trait Analysis (GCTA) (Yang et al. 2011) was used to compute the variance in the ADHD symptom score explained by common SNPs in the two largest cohorts included in the SAGA meta-analysis, the NTR and NESDA (n > 1500 unrelated subjects, we selected one random sibling per twin pair across all pairs). A genetic relationship matrix (GRM) for all individuals in the dataset was estimated based on SNPs with high imputation quality (see Supplementary Methods). Bivariate GCTA (Yang et al. 2011) was additionally run on the CAARS ADHD-index and ASR-ADHD data also available in the NTR cohort, to assess the genetic correlation (r_g) between the two diagnostic instruments.

Genome-wide association and meta-analysis in the SAGA consortium

GWAS was conducted in each cohort by linear regression under an additive model. Age was included as a covariate, but not gender, which was not significantly associated with the ADHD scores in any study. Four principal components were added to account for possible population stratification effects. Information on software packages is provided in Supplementary Table 1. In all analyses, the uncertainty of the imputed genotypes was taken into account depending on the genotyping array and imputation software (threshold used: $0.8 < \text{INFO} < 1.1$, $\text{RSQR} \geq 0.6$, $\text{INFO} \geq 0.6$; see Supplementary Table 1). Location of SNPs reported is from the build 37 (hg19) 1000G data. Meta-analysis was conducted in METAL (http://www.sph.umich.edu/csg/abecasis/metal/index.html) by the *p*-value-based method, given the intrinsic variability of the quantitative traits used that limits our ability to combine betas and standard errors freely. While the trait scores used in the different GWAS are correlated, we cannot be completely sure that the sampling distribution of these traits is exactly the same (see Supplementary Methods). The meta-analysis was performed in the full sample (nine cohorts) and restricted to the population-based samples (seven cohorts; "restricted sample").

Replication in the EAGLE consortium

Within EAGLE, association of ADHD-related measures was assessed in nine population-based childhood cohorts with genotype data imputed against the 1000 Genomes reference panel (Middeldorp et al. 2016). Linear regression of the phenotype on sex, age, genotype dose, and principal components was performed in all cohorts, followed by meta-analysis based on *p*-values in METAL. The TRAILS cohort is part of both consortia, and was excluded from the EAGLE consortium for replication analysis, leaving a total of 14,776 children from eight cohorts.

Lookup of significant GWAS Loci

Evidence for an effect of the 12 independent ADHD-associated SNPs from the PGC + iPSYCH GWASMA on adult ADHD symptoms was studied through a look-up of results. Linkage disequilibrium (LD)-independent loci with corresponding index-SNPs were obtained from Table 1 of Demontis et al. (2018). If the index variant was not present in the SAGA data set, a proxy variant was selected using LDlink (https://analysistools.nci.nih.gov/LDlink/). The Bonferroni-corrected significance level was set at $p = 0.05/12 = 0.00417$.

Linkage disequilibrium score regression (LDSR) analysis

LDSR was used to estimate the genetic correlation between the PGC + iPSYCH sample of children (Demontis et al. 2018) the SAGA sample of adults and the ADHD GWASMA data of the EAGLE consortium. Each dataset underwent additional filtering for markers overlapping with HapMap Project Phase 3 SNPs, INFO score ≥ 0.9 (where available), and MAF ≥ 1%. Indels and strand-ambiguous SNPs were removed. LDSR analysis was performed using the LDSR package (https://github.com/bulik/ldsc (Bulik-Sullivan et al. 2015), see Supplementary Methods).

Gene-based analysis of common and rare variants

Genes containing SNPs with *p*-values $< 1 \times 10^{-6}$ in the meta-analysis of the nine cohorts were selected for gene-based tests using common and rare variants. The common variant

analysis was performed in MAGMA (de Leeuw et al. 2015). Flanking regions of 25 kb for each gene were included in the analyses. The rare variant analysis was performed with the sequence kernel association test (SKAT; only in the ERF study) library of the R-software (Wu et al. 2011). Only characterized genes plus the STXBP5-AS1 gene were analyzed.

Functional analyses

Follow-up functional analyses were performed on the STXBP5-AS1 locus containing the best association *p*-value in the SAGA full sample GWASMA. Given that the *STXBP5-AS1* gene, which contains the top hits, is hitherto uncharacterized, we investigated its function. *STXBP5-AS1* encodes a long noncoding RNA (lncRNA). Although human *STXBP5-AS1* does not have any orthologues listed in the UniGene database, it is conserved in primates and shows a modest conservation in rodents (Supplementary Table 7 and alignment in Supplemental Fig. 2). In the hg19 genome release annotation *STXBP5-AS1* is located next to *STXBP5* in the opposite orientation, with antisense sequence overlap in exon one of *STXBP5* (Fig. 1a). It may be hypothesized that *STXBP5-AS1* affects *STXBP5* expression. For such natural antisense RNAs, both repression and enhancement of the expression of cognate genes have been described (Kimura et al. 2013; Matsui et al. 2008). We tested this hypothesis by designing a reporter gene fusing exon 1 of STXBP5 to EGFP, and quantifying its expression in human HEK293 cells. Human *STXBP5-AS1* encodes multiple splice variants, many of which lack a region that overlaps the *STXBP5* gene. To test for regulatory effects of *STXBP5-AS1* on the expression of *STXBP5*, a fluorescent reporter construct was designed to contain the region of antisense overlap (see Supplementary Methods).

Mouse models

According to current annotation in GRCm38.p6, the mouse lncRNA gene *Gm28905* shares sequence similarity with human *STXBP5-AS1* but lacks antisense overlap with mouse *Stxbp5*. Nonetheless, we use the annotation *Stxbp5-AS1* next to the official gene name. Given the in vitro effects

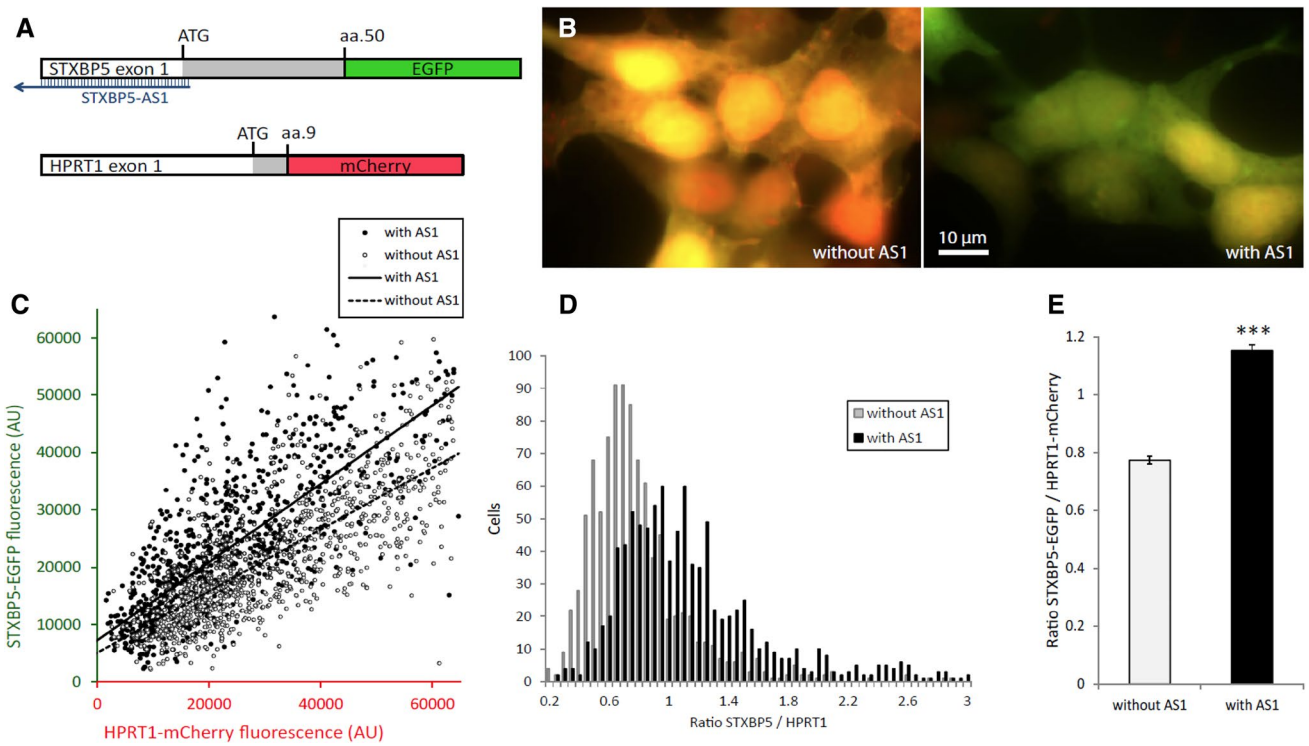

Fig. 1 STXBP5-AS1 positively regulates the expression of its cognate mRNA. **a** *Top*: Design of two reporter constructs. Top: Exon 1 of human *STXBP5*, containing the natural 5′UTR and encoding the first 50 amino acids, was fused in-frame to EGFP. The *STXBP5-AS1* transcript including the region showing perfect (100%) sequence overlap with the encoded *STXBP5* transcript is depicted schematically in blue. *Bottom*: To control for transfection efficiency and differences in cellular metabolic rates, we co-expressed a non-target mRNA comprised of human *HPRT1* exon 1 fused to mCherry. **b** Typical examples of HEK293 cells expressing both constructs with or without STXBP5-AS1. **c** Quantitation of EGFP and mCherry fluorescence in presence or absence of STXBP5-AS1 (947 and 974 cells respectively). **d, e** The ratio of STXBP5-EGFP and HPRT1-mCherry was calculated for each cell. Data are presented as a histogram **d** or as mean ± SEM **e**. ***$p = 6 \times 10^{-51}$; $t_{946} = 4.4412$, Student's *t*-test

on STXBP5-EGFP protein expression, we tested the relationship between gene expression of both mouse *Stxbp5* and/or *Gm28905* / *Stxbp5-AS1* and measures of behavioral impulsivity. We analyzed gene expression in medial prefrontal cortex of three mouse inbred strains previously described to have large differences in motor impulsivity (Loos et al. 2014). RNA was derived from prefrontal cortex of adult male mice from the inbred strain C57BL/6J (n = 8) and recombinant inbred strains BXD29 (n = 8) and BXD68 (n = 7), and gene expression was quantitated (see Supplementary Methods). For one C57BL/6J animal the RNA isolation failed, and for one BXD68 animal we did not have behavioral data; hence, the n-numbers vary between analyses. Strains were bred in the facility of the Neuro-Bsik consortium of the VU University (Amsterdam, The Netherlands) and used for behavioral analyses (Loos et al. 2014; Spijker et al. 2004).

Results

ADHD symptom scores phenotypic characterization

Quantitative assessment instruments are listed in Table 1. The quantitative phenotypes showed a weak, negative correlation with age and no association with gender in any cohort. We estimated the phenotypic correlation between the CAARS ADHD-index and the ASR-ADHD in the NTR (n = 15,226; average age 40 years, SD = 16.1) to be 0.67 ($p < 0.0001$). In younger participants in the age range of the TRAILS cohort (18–22 years, n = 2687), the correlation was similar (r = 0.68, $p < 0.0001$).

Genetic correlations of ADHD symptom scores

A significant SNP-based heritability was estimated for the CAARS ADHD-index in a subsample of each of the two largest cohorts: 30% (SE = 16.7%, $p = 0.035$) in NESDA (n = 1841 unrelated subjects) and 15% (SE = 7.8%, $p = 0.020$) in NTR (n = 3881 unrelated participants). Both estimates are significant, but given the sample sizes in each cohort, they are not statistically different from each other. We also estimated the genetic correlation for the CAARS ADHD-index and the ASR-ADHD using bivariate GCTA. In all individuals from the NTR with genotype and phenotype data (n = 6036 related and unrelated subjects), the genetic correlation was 0.818 (SE = 0.256). When analyzing the bivariate data in 2921 unrelated subjects, the point estimate of the genetic correlation was 0.813 (SE = 0.364).

We estimated the genetic correlation between PGC + iPSYCH and the complete SAGA sample to be 0.541 (SE = 0.447, $p = 2.26 \times 10^{-1}$; the VHIR cohort present in both studies). The SNP-based heritability of the complete SAGA samples, assessed by LDSR, was 0.0183 (SE = 0.038). The genetic correlation between the complete SAGA sample and the childhood ADHD cohort of the EAGLE-ADHD consortium was modest and non-significant ($r_g = 0.2959$, SE = 1.2906, $p = 0.8187$).

In NTR and NESDA, a subset of participants (n = 6678) had additional phenotype data on hyperactivity/impulsivity and inattention symptom subscales of the CAARS available. These scales of each nine items are non-overlapping with the 12 ADHD-index items (see Supplementary Table 8). For hyperactivity/impulsivity symptoms, the *p*-value for association with rs12661753 was 1.51×10^{-5}, whereas for inattention it was 3.53×10^{-2}, suggesting a differential effect of the variant between hyperactivity and impulsivity but we cannot claim that this is a significant difference between the two traits.

Genome-wide association, meta-analysis and replication

For the nine separate GWAS (in the SAGA Consortium), the genomic control inflation factors (lambda) ranged between 0.960 and 1.027 (mean lambda 0.999, Supplementary Table 2). Meta-analysis (Fig. 2a) of the full sample revealed the lowest *p*-value (3.02×10^{-7}) for the intronic SNP rs12661753 in *STXBP5-AS1* (Supplementary Fig. 3E); for the meta-analysis of the restricted sample, *p*-value for this SNP was 1.48×10^{-6} (Fig. 2b). Replication was observed for rs12661753 ($p = 3.07 \times 10^{-2}$) for childhood ADHD symptoms in the EAGLE-ADHD consortium (Middeldorp et al. 2016). The subsequent GWASMA between SAGA and EAGLE revealed the best association *p*-value = 2.05×10^{-7} for SNP rs12664716 (n = 29,465; Supplementary Fig. 3F) located in the *STXBP5-AS1* gene, and in high LD ($D' = 1.0$, $r^2 = 0.98$) with rs12661753 ($P_{SAGA-EAGLE} = 3.55 \times 10^{-7}$; Fig. 2c).

The index variant rs12661753 was not associated with ADHD risk in the recent case-control PGC + iPSYCH GWASMA of ADHD in a sample mainly consisting of children ($p = 0.6316$, n = 55,374). A look-up of genome-wide significant ADHD index SNPs from this PGC + iPSYCH GWASMA for association in the SAGA consortium also revealed no significant associations with adult ADHD symptoms (Supplementary Table 6).

As shown in Table 2 and Supplementary Table 3, eight independent clumped loci (including 50 common variants) showed *p*-values $< 1 \times 10^{-6}$. Of these, four were also amongst the top-associated loci from the restricted SAGA GWASMA (no patients; Supplementary Table 4). The genes closest to these SNPs were selected for gene-based analysis (Table 2). Analysis of common variants in seven genes (plus 25 kb flanking regions) in the SAGA GWASMA showed significant association with ADHD symptoms. Three significant

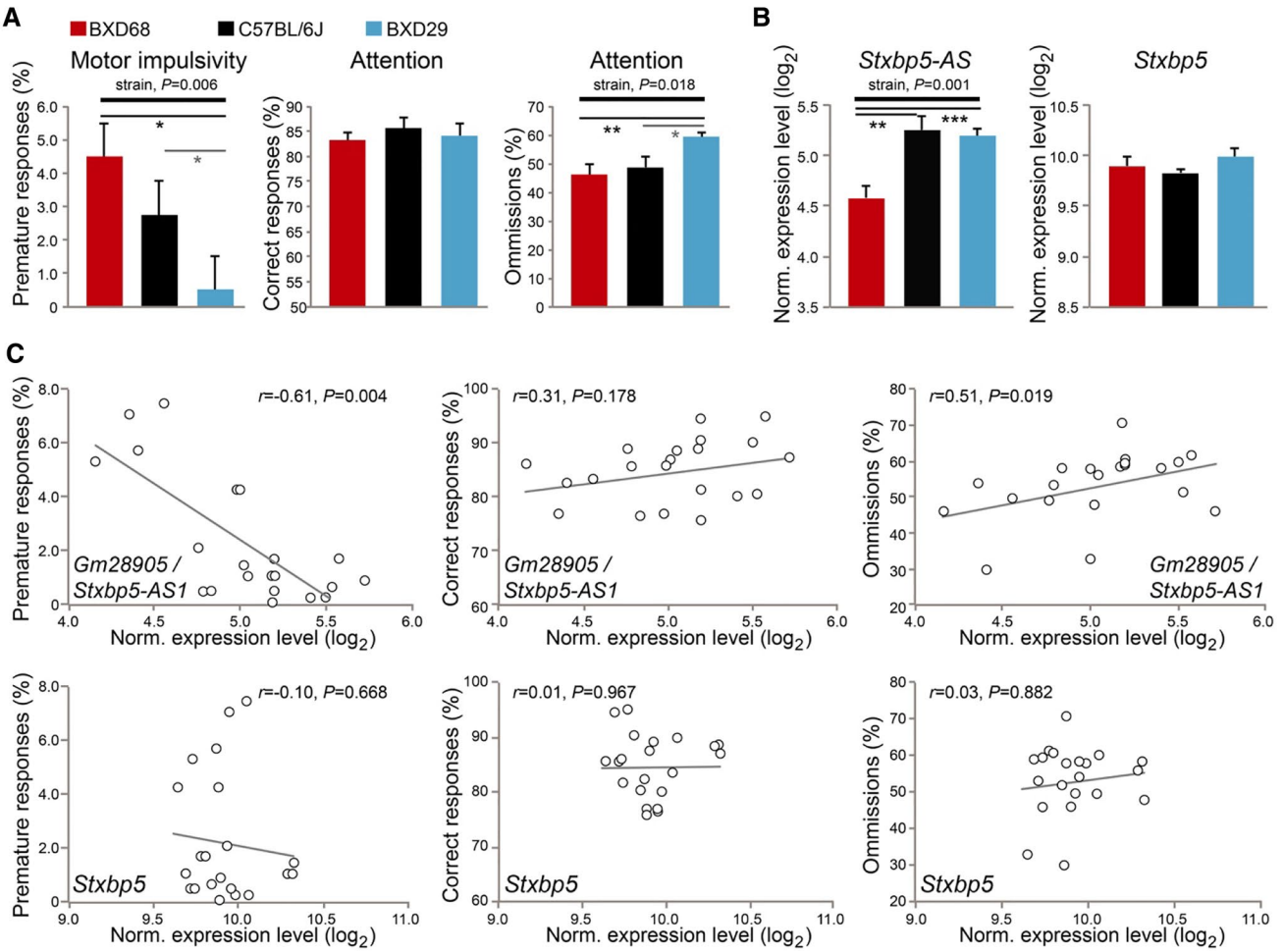

Fig. 2 Manhattan & QQ plot of the ADHD Symptom Total Score meta-analysis from the complete SAGA consortium (Panel a), without patients (Panel b) and meta-analysis from the SAGA & EAGLE consortia (Panel c). **a** Manhattan & QQ plot of the ADHD symptom total score meta-analysis from the complete SAGA consortium. Association *p*-values > 0.05 in the GWAS plot are not shown. All *p*-values are shown in the QQ-plot. **b** Manhattan & QQ plot of the ADHD Symptom Total Score meta-analysis from the SAGA consortium without patient cohorts. Association *p*-values > 0.05 in the GWAS plot are not shown. All *p*-values are shown in the QQ-plot. **c** Manhattan & QQ plot of the ADHD Symptom Total Score meta-analysis from the SAGA & EAGLE consortia

Table 2 Most strongly associated (clumped) SNPs (p-value $< 1 \times 10^{-6}$) coming from the meta-analysis of nine cohorts from the SAGA consortium in physical position order (hg19)

SNP name	Chr	Locus	Pos*	*p*-Value	Tested/Non-tested Allele	Frequency tested Allele	Gene(s) in locus
rs12661753	6	6q24.3	147409235	3.02×10^{-7}	A/G	0.962	*STXBP5-AS1*
rs1564034	2	2p25.2	6510305	2.15×10^{-6}	T/G	0.670	*LINC01247*
rs1930272	1	1p31.1	83491910	4.75×10^{-6}	T/C	0.544	*LOC107985037*
rs28734069	4	4q26	120042409	5.77×10^{-6}	T/C	0.016	*LOC102723967; LOC105377395*
rs13274695	8	8p23.2	3723378	6.00×10^{-6}	A/G	0.077	*CSMD1; LOC105377790*
rs73204517	13	13q21.33	69920315	7.19×10^{-6}	C/G	0.126	Downstream *LINC00383*
rs11209188	1	1p31.3	68455306	7.88×10^{-6}	A/G	0.534	*GNG12-AS1*
rs2189255	8	8q21.3	91190297	9.61×10^{-6}	T/C	0.703	*CALB1; LINC00534*

SNPs organized on association *p*-values. *bp position based on the GRCh37.p13 build; Allele frequency of tested allele based on n = 14,689

findings ($p < 0.007$) were for the *STXBP5-AS1, LINC01247* and the *LINC00534* genes. Nominal significant associations ($p < 0.05$ gene-based) were seen for, *CALB1, GNG12-AS1, STXBP5* (Supplementary Table 5). It is important to note that *STXBP5* and *STXBP5-AS1* have no physical separation, thus their 25 kb flanking regions overlap (STXBP5 is located on the forward ["+"] and STXBP5-AS1 on the reverse ["-"] strand—see Supplementary Figs. 3E, F). The rare variant analysis also showed nominal association for *STXBP5*. For four genes (*GNG12-AS1, LINC01247, STXBP5-AS1, LINC00534)*, rare variants were not observed/detected (Supplementary Table 5).

Functional analysis

Expression of the antisense lncRNA variant *STXBP5-AS1-003* (containing the overlap with *STXBP5*) caused an increase in the fluorescence ratio between STXBP5-EGFP and the control (Fig. 1b–e).

Mouse models

We confirmed the recombinant inbred (RI) strain difference in motor impulsivity between the BXD68, BXD29, and C57BL/6J strains (Kruskal–Wallis, $p = 0.006$), measured as premature responses in the 5-choice serial reaction time task (Loos et al. 2014). In addition, these RI strains showed differences in the attention parameter of errors of omission ($F_{2,19} = 4.98$, $p = 0.018$), but not the attention parameter of percentage correct responses ($F_{2,19} = 0.32$, $p = 0.733$) (Fig. 3a). In these mice, we detected expression of *Gm28905 / Stxbp5-AS1* lncRNA in the prefrontal cortex by real-time quantitative PCR, which differed across strains ($F_{2,19} = 11.53$; $p = 0.001$). This transcript showed lowest expression in the most highly impulsive strain, BXD68 (BXD68: 4.58 ± 0.11, C57BL/6J: 5.25 ± 0.14, BXD29: 5.19 ± 0.07, Bonferroni-corrected post-hoc $P_{BXD68\ vs\ C57BL/6J} = 0.001$; $P_{BXD68\ vs\ BXD29} = 0.002$) (Fig. 3b). Expression of *Stxbp5* mRNA was not different between the three strains (BXD68: 9.89 ± 0.09; C57BL/6J: 9.83 ± 0.04; BXD29: 9.99 ± 0.08; $F_{2,19} = 1.23$; $p = 0.314$). These results suggest that *Gm28905 / Stxbp5-AS1* affects impulsivity independent of the *Stxbp5* transcript. Examining correlations between *Gm28905 / Stxbp5-AS1* transcript level and impulsivity/(in)attention measures, we found a significant correlation with motor impulsivity (Spearman's rho = −0.61; $p = 0.004$; BCa −0.15 to −0.85) that withstood Bonferroni correction (threshold p-value < 0.008). A nominally significant association was found for expression of *Gm28905/Stxbp5-AS1* with attention, when measured as errors of omission (Guillem et al. 2011) (Spearman's rho = 0.51; $p = 0.019$; BCa 0.01–0.86), but not when measured as percentage correct responses (Pearson $r = 0.31$; $p = 0.178$; BCa −0.08–0.61). Expression of *Stxbp5* did not correlate with any of these parameters (Fig. 3c).

Discussion

We report a genome-wide association meta-analysis with three different but correlated adult ADHD symptom lists in nine European adult population-based and case-only cohorts (n = 14,689 individuals). The *STXBP5-AS1* locus (best SNP $p = 3.02 \times 10^{-7}$) was the most strongly associated in this meta-analysis. This association was replicated in the EAGLE meta-analysis ($P_{EAGLE} = 2.89 \times 10^{-2}$), and the top-hit from the full SAGA-EAGLE GWASMA was also located in the *STXBP5-AS1* gene and in almost perfect LD with the original finding (SNP rs12664716, $P_{SAGA-EAGLE} = 2.05 \times 10^{-7}$; n = 29,465).

For the adult ADHD-index, an earlier large twin family study estimated total h^2 at 30%. For clinically assessed adult ADHD as well as for total sum scores for Attention Problems the h^2 estimates were even higher, as summarized in the introduction. Common SNPs thus are hypothesized to contain substantial information concerning the genetic variance underlying adult ADHD and Attention Problems. The SNP-based heritability analyses, which were ran prior to GWASMA, provided estimates of 15–30% explained variance of adult ADHD symptom scores in the general population. Such estimates are comparable with the estimates obtained for ADHD and four additional categorically defined psychiatric disorders (Psychiatric Genomics Consortium et al. 2013), providing rationale for a gene-finding enterprise for adult ADHD symptoms in the general population. The significant SNP-based heritability and the considerable phenotypic and genetic correlations between assessment instruments support the validity of our meta-analysis approach of GWA results obtained across contributing data sets.

The function of the *STXBP5-AS1*-encoded lncRNA is currently unknown. *STXBP5-AS1* has been proposed as a prognostic biomarker for survival of cancer patients (Guo et al. 2016), but no information is available for its role in ADHD, related traits, or other psychiatric diseases. In humans it overlaps in anti-sense with *STXBP5*, encoding a protein involved in synaptic function by regulating neurotransmitter release through stimulating SNARE complex formation (Sakisaka et al. 2008; Yizhar et al. 2004). This complex plays a major role in intracellular vesicular trafficking in eukaryotic cells and is involved in the exocytotic release of neurotransmitters during synaptic transmission (Antonucci et al. 2016). Genes related to the SNARE complex and its regulators have been investigated in ADHD (Bonvicini et al. 2016), and current results suggest that this complex may exert distinct roles throughout development, with age-specific effects of its genetic variants on ADHD

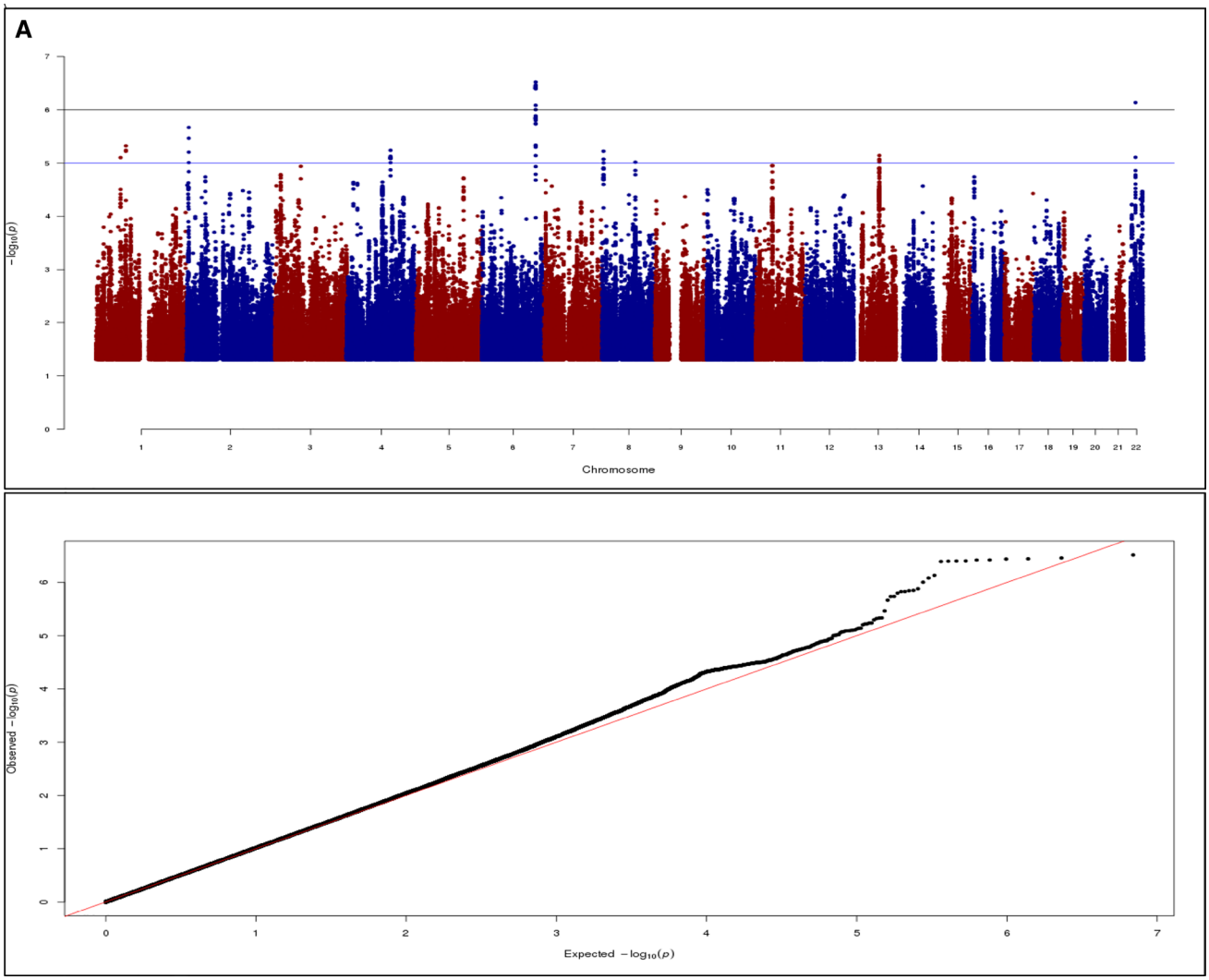

Fig. 3 Prefrontal cortex gene expression of mouse *Gm28905 / Stxbp5-AS1* is correlated with impulsivity. **a** Mouse RI strains BXD68 (red, n = 6), C57BL/6J (black, n = 8) and BXD29 (blue, n = 8) were selected based on a difference in premature responses (motor impulsivity; Bonferroni-corrected post-hoc BXD68 *vs.* BXD29, $p = 0.005$; C57BL/6J *vs.* BXD29, $p = 0.122$) and the attention parameter of error of omissions (Bonferroni-corrected post-hoc BXD68 *vs.* BXD29, $p = 0.031$; C57BL/6J *vs.* BXD29, $p = 0.063$), without being different on the attention parameter percentage correct responses. Shown are data (mean ± SEM) of the animals used for gene expression analysis (see **b** and material & methods). **b** Strain mean ± SEM of prefrontal cortex gene expression in BXD68 (red, n = 7), C57 (black, n = 7), and BXD29 (blue, n = 8) for *Gm28905 / Stxbp5-AS1* (left) and *Stxbp5* (right). *Gm28905 / Stxbp5-AS1* is differentially expressed between strains, with lower expression in BXD68. Yet, *Stxbp5* shows no differential expression. No difference in variation was observed. **c** Gene expression of *Gm28905 / Stxbp5-AS1* (upper panels) in individual mice for which behavioral data was available (BXD68, n = 6; C57BL/6J, n = 7; BXD29, n = 8) correlated well with premature responses (motor impulsivity; left), not with accuracy (attention; middle), and showed a trend towards correlation with errors of omissions (attention; right). *Stxbp5* expression (lower panels) did not correlate with any of these parameters. Spearman's rho (motor impulsivity, error of omissions) and Pearson's *r*-values (percentage correct responses) are given, as well as uncorrected *p*-values; note that the Bonferroni-corrected threshold is $p < 0.008$. Trend lines are given in gray. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$ for post-hoc t-tests, where a gray line and asterisk indicates a nominal significance that did not survive Bonferroni correction

behavior (Cupertino et al. 2016). Specifically, deletions and mutations of *STXBP5* occur in autism (Davis et al. 2009) and epilepsy (Dhillon et al. 2011). *STXBP5* has a presynaptic role that negatively regulates neurotransmitter release by forming syntaxin-SNAP25-tomosyn complex (Sakisaka et al. 2004). However, the postsynaptic role of STXBP5 has not been elucidated well.

Post-hoc analysis suggested that *STXBP5-AS1* could significantly affect both hyperactivity-impulsivity and inattention. We were able to determine that both hyperactivity/

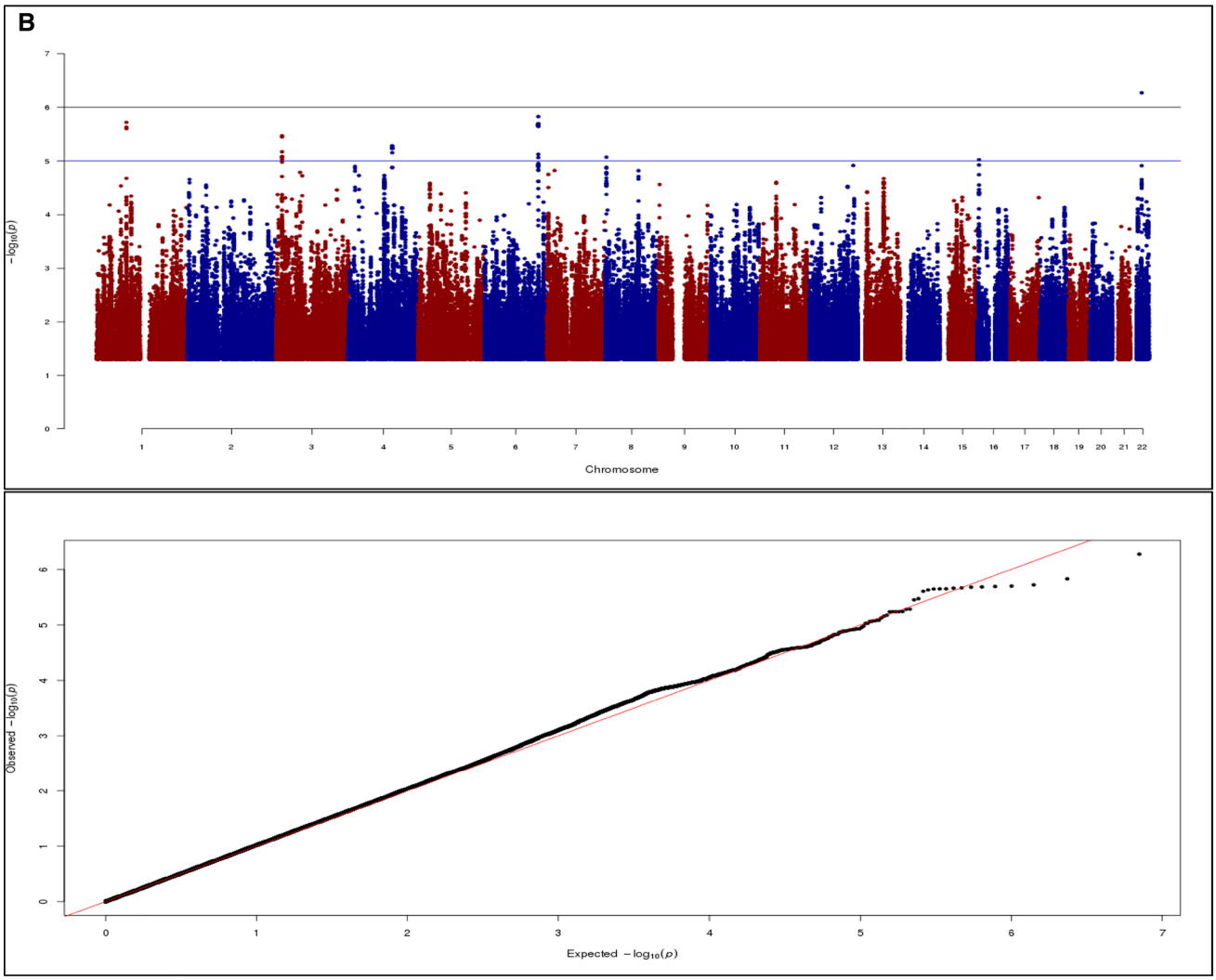

Fig. 3 (continued)

impulsivity and inattention symptoms were significantly associated with rs12661753 ($p = 1.51 \times 10^{-5}$, $p = 3.53 \times 10^{-2}$, respectively). Even though the effect size is larger for hyperactivity, we cannot claim that this is a significant difference (Supplemental Fig. 1). Our experiments in HEK293 cells showed that the lncRNA does not cause antisense inhibition of *STXBP5, but may enhance STXBP5 expression*. The role of *STXBP5-AS1* with impulsivity was corroborated in behavioral studies in mice. The unchanged *Stxbp5* mRNA levels in mouse strains expressing different *Gm28905* levels is consistent with the current annotation that lack antisense overlap, and suggests that the lncRNA might contribute to impulsivity by a Stxbp5-independent mechanism. In line with this idea, *Gm28905* expression (but not that of *Stxbp5*) correlated negatively with motor impulsivity in mice.

Our study should be viewed in the light of some strengths and limitations. A clear strength was the functional analyses that provided a likely candidate associated with adult and childhood ADHD symptoms. A limitation of our study was the limited sample size in the SAGA meta-analysis in combination with the use of different phenotyping instruments in the cohorts. Our results did not replicate the findings from the latest GWASMA on ADHD cases and controls in children (Demontis et al. 2018). Nevertheless, we were able to detect a strong association signal that (i) was also found associated in the EAGLE GWASMA (albeit less strong but in a pediatric population-based data) and (ii) shows a robust functional effect in two independent functional studies corrected for multiple testing, as indicated on page five of the Supplementary Methods, (p-value of $5 \times 10 - 2/6 = 0.0083$; correlations of two transcripts were tested for three parameters).

We did see some heterogeneity in the meta analysis result for SNP rs12661753 (Supplementary Table 3). This is partly due to the combination of the between-study heterogeneity of the measures, even for the higher correlated phenotypes,

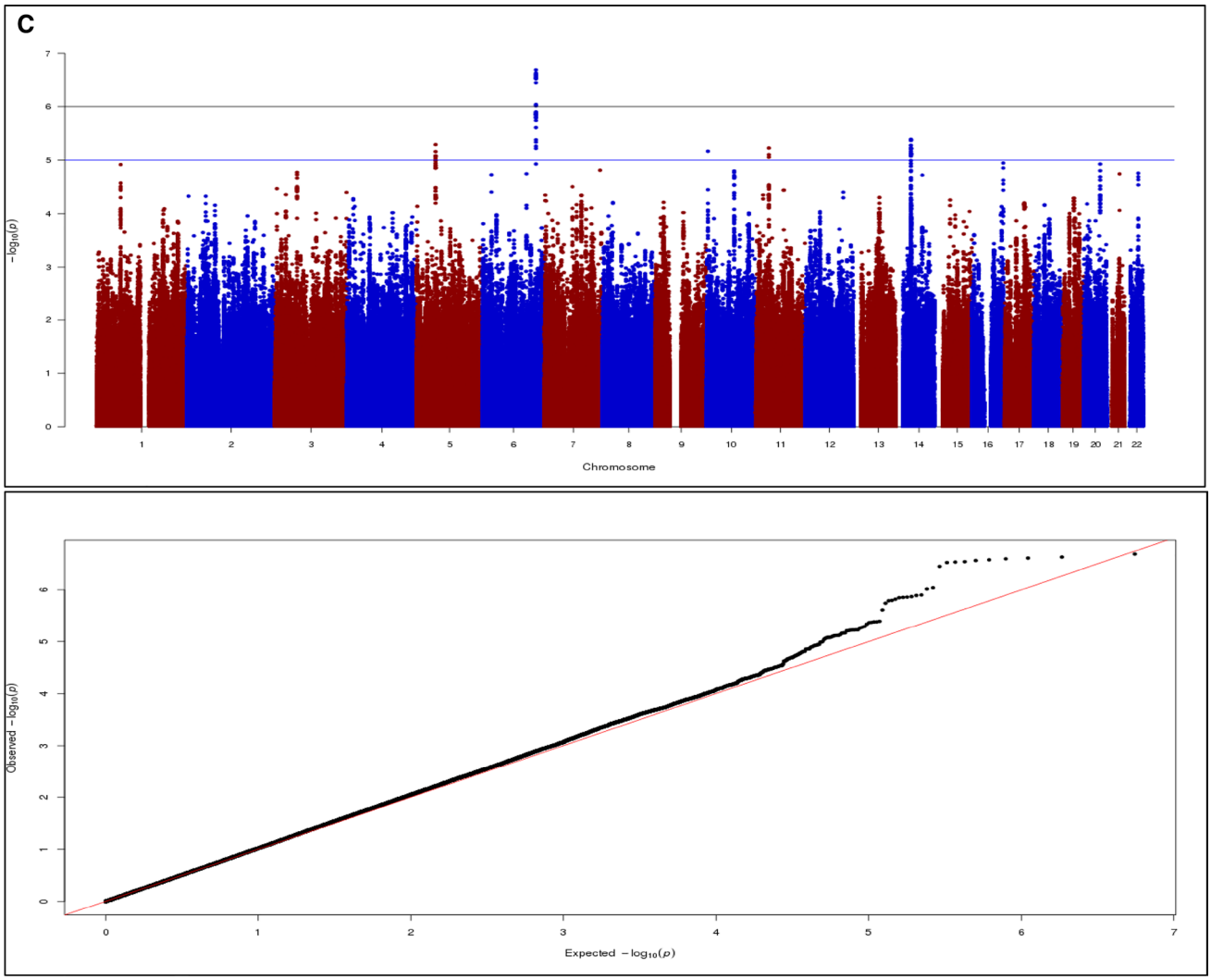

Fig. 3 (continued)

the inclusion of small studies that can skew the true variances of the investigated traits and a (somewhat) low(er) frequency of the tested allele of the best associated SNP (mean MAF = 0.038; range MAF = 0.013–0.049; for details on each cohort see Supplementary Table 9). The aim of our study was to identify ADHD related genes in adults by using the quantitative ADHD symptom scores provided by both population-based and case-control samples. This means that some compromise had to be achieved when combining this information. We took care of reducing the noise by (i) performing individual GWAS (per cohort, per trait) and then meta analyzing the results (which maximizes information per study including the smaller ones) and (ii) profiting from the strong phenotypic and genetic correlations between the instruments, which might have helped us maintain a better power to detect true association signals. Nevertheless, while our sample is not small (~ 15 k participants), in order to detect genome wide significant signals, sample sizes need to increase.

The genetic correlation of PCG + iPSYCH with SAGA should be interpreted carefully because the standard error was high. The fact that the PCG + iPSYCH/SAGA r_g (0.54; SE = 0.447) did not differ from the published r_g estimate between the PCG + iPSYCH GWASMA and a GWAS of the 23 andMe sample (0.65, SE = 0.114) (Demontis et al. 2018) is encouraging. These correlations would confirm the genetic stability of ADHD in childhood and adulthood, as was also suggested from longitudinal modeling of twin data (Kan et al. 2013). The estimated genetic correlation between the 23 andMe and PGC + iPSYCH analyses was significant but lower than the genetic correlation of the EAGLE and PCG + iPSYCH childhood cohorts ($r_g = 0.943$, SE = 0.204, $p = 3.65 \times 10^{-6}$) (Demontis et al. 2018). The ADHD diagnosis (yes/no) in 23 andMe is based on the self-reported answer

to a single question about presence of a lifetime diagnosis of ADHD (Demontis et al. 2018) and we do not know if the 23 andMe participants were diagnosed in childhood or as adults. Also, the modest and non-significant genetic correlation between the SAGA and the EAGLE samples should be interpreted cautiously. For both samples, the SNP-based heritability as estimated by LDSC was low and both samples may suffer from their phenotypic heterogeneity as well as limited sample size. A further increase in GWAS sample size updated r_g results is still needed. These results could help us understand the (seemingly) different genetic correlation patterns between the association results estimated from the GWAS of adult (population-based) ADHD behavior and the GWAS from children. At this point, the lack of power makes these analyses inconclusive.

Our study shows that self-reported adult ADHD symptoms measured in the general population have a genetic component and that performing population-based GWASMA of adult ADHD symptoms provides novel insights into the genetic underpinnings of hyperactivity/impulsivity symptoms that are a hallmark of ADHD. We were able to carry out functional follow-up studies which considerably strengthened our findings for a possible role of *STXBP5-AS1* and its mouse ortholog *Gm28905* in ADHD symptom etiology.

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Compliance with ethical standards

Conflict of interest J.K.B. has been in the past 3 years a consultant to/member of advisory board of/and/or speaker for Janssen Cilag BV, Eli Lilly, Shire, Lundbeck, Roche and Servier. He is not an employee of any of these companies, and not a stock shareholder of any of these companies. He has no other financial or material support, including expert testimony, patents, royalties. J.J.S.K. has been a speaker for Eli Lilly, Janssen and Shire until 2012, and received unrestricted research grants in 2010 from Janssen and Shire. J.A.R.Q. was on the speakers' bureau and/or acted as consultant for Eli-Lilly, Janssen-Cilag, Novartis, Shire, Lundbeck, Ferrer and Rubió in the last 3 years. He also received travel awards (air tickets + hotel) for taking part in psychiatric meetings from Janssen-Cilag, Rubió, Shire and Eli-Lilly. The ADHD Program chaired by him received unrestricted educational and research support from the following pharmaceutical companies in the last 3 years: Eli-Lilly, Janssen-Cilag, Shire, Rovi and Rubió. B.F. received educational speaking fees from Shire and Medice. A.A.V., A.J.G., H.H.M.D., M.K., D.V., S.S., T.E.G., J.J.H., P.J.vdM., V.M. K, R.P., I.M.N., B.W.J.H.P., I.O.F, A.dB., C.M.vD., P.J.H., L.A.K., M.H, M.K., C.M.M., K.G.O., S.H.V., C.S.M., M.R., C.A.H., N.A., A.B.S., D.I.B. report no conflict of interest.

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